

## Isolation of *Campylobacter fetus* subsp. *jejuni* from Migratory Waterfowl

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Since the sources from which humans acquire *Campylobacter* enteritis are only partially known, we studied the frequency of carriage of *Campylobacter fetus* subsp. *jejuni* in migratory waterfowl. Cecal contents of various species of wild ducks were cultured on selective media that contained antibiotics to inhibit normal flora. Thirty-five percent of the 445 ducks cultured harbored *C. fetus* subsp. *jejuni*. Migratory waterfowl are yet another reservoir for this enteric pathogen and may be of public health importance for humans in the contamination of water or when used as food.

*Campylobacter fetus* subsp. *jejuni* has been recognized only recently as a human enteric pathogen. Selective methods of culture have enabled isolation of this agent in 3 to 14% of cases of acute diarrheal illness in humans (1, 4, 13, 15). Despite its apparent public health importance, however, the natural reservoirs and sources of *Campylobacter* for human infections have not yet been fully determined.

Among domestic animals, *C. fetus* subsp. *jejuni* is known to cause abortion in sheep (17), winter scours in cattle (9), infectious hepatitis in chickens (17), and possibly enteritis in dogs (2). In wild animal populations, it has been isolated from bank voles (6), pigeons, blackbirds, starlings, and sparrows (16). The objective of the present study was to determine the frequency of *C. fetus* subsp. *jejuni* in migratory waterfowl. The observed carrier rate of 35% may make these waterfowl a potential health hazard for humans.

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### MATERIALS AND METHODS

From November 1978 through January 1980, 445 wild ducks were collected by cooperating hunters in northern Colorado. The waterfowl were cultured immediately in the field or were transported to the laboratory within 24 h at subfreezing temperatures. Ceca were opened under sterile conditions and samples of cecal contents were collected with sterile swabs. In 64 of the ducks, cloacal swabs were obtained along with cecal specimens to compare the isolation rates

from these two sources.

The cecal and cloacal swabs were inoculated on a selective plate medium that consisted of brucella agar base (BBL Microbiology Systems, Cockeysville, Md.), 5% sheep blood, and the following antimicrobials, per liter: amphotericin B, 2 mg; cephalothin, 15 mg; polymyxin B, 2,500 U; trimethoprim, 5 mg; and vancomycin, 10 mg (3). Plates were incubated for 48 h at 42°C in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen. Typical flat gray watery nonhemolytic spreading colonies were Gram stained (counterstained with a 0.8% solution of carbol fuchsin), and those colonies consisting of small gram-negative curved or spiral rods were further isolated. Identification of *C. fetus* subsp. *jejuni* was based on typical vibrio forms, colony characteristics, oxidase and catalase positivity, darting motility on dark-field examination, lack of fermentation or oxidation of glucose, production of hydrogen sulfide as detected by lead acetate paper over Kligler slants, growth at 42°C but not at 25°C, and sensitivity to nalidixic acid (30-μg disks) (1). Isolates were cultured only under microaerophilic conditions, and no attempt was made to test growth in other atmospheres such as 21% oxygen.

Statistical comparisons were made with the chi-square test. Paired data for cecal versus cloacal specimens were analyzed with the McNemar test for correlated proportions (14).

### RESULTS

Of the 445 migratory waterfowl studied, 154 (35%) carried *C. fetus* subsp. *jejuni*. The frequency of isolation among different species is shown in Table 1. Shovelers had significantly higher ( $P < 0.001$ ) and green-winged teal had significantly lower ( $P < 0.01$ ) isolation rates than the remaining ducks. Gadwalls had a low isolation rate (15%), but the numbers were too small to achieve statistical significance. The 17 ducks

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TABLE 1. Isolation of *C. fetus* subsp. *jejuni* from cecal contents of various species of migratory waterfowl

Species of waterfowl	No. tested	No. positive	%
Shoveler ( <i>Spatula clypeata</i> )	35	23	66
Pintail ( <i>Anas acuta</i> )	30	15	50
American widgeon ( <i>Mareca americana</i> )	38	16	42
Mallard ( <i>Anas platyrhynchos</i> )	243	82	34
Green-winged teal ( <i>Anas carolinensis</i> )	56	9	16
Gadwall ( <i>Anas strepera</i> )	26	4	15
Other species	17	5	29
Totals	445	154	35

of "other" species collected in numbers too small for separate statistical analysis included redhead (*Aythya americana*), bufflehead (*Bucephala albeola*), and lesser scaup (*Aythya affinis*). Carrier rates of hens compared with drakes were similar for all species, with the exception of mallard hens which had a significantly higher carrier rate of *C. fetus* subsp. *jejuni* than did mallard drakes (50 versus 31%,  $P < 0.05$ ).

There was no significant difference in carrier rates between resident Colorado ducks collected in October (33%) and migrating northern ducks collected later in the season (35%). Carrier rates for all species were similar from one year to the next.

A comparison of culture techniques in 64 ducks showed that *C. fetus* subsp. *jejuni* was isolated from both cecal and cloacal swabs in 9 ducks, from only cecal swabs in 11 ducks, and from only the cloacal swab in 1 duck. The higher rate of isolation with cecal swabs was statistically significant ( $P < 0.001$ ).

## DISCUSSION

The north central plateau of Colorado east of the Rocky Mountains is part of the Central Flyway for migratory birds. Its many rivers, streams, warm water sloughs, ponds, shallow reservoirs, and abundant fields of grain attract hundreds of thousands of migratory ducks yearly, with estimated peak daily counts reaching over 200,000 in the Platteville area during the 1978 and 1979 hunting seasons (Colorado State Fish and Game Department, personal communication). This study showed that 35% of a sample of 445 of these migratory waterfowl carried *C. fetus* subsp. *jejuni*.

The species variation noted in isolation rates may be related to differences in feeding habits. The lowest carriage rates were found in gadwalls

(15%) and green-winged teal (16%), which feed almost exclusively on vegetable matter. In contrast, shoveler ducks, which had a 66% prevalence of *C. fetus* subsp. *jejuni*, often strain mud from the bottom of ponds and rivers to extract molluscs and other animal foods that constitute approximately one-third of their diet (11). The animal food intake, as well as the isolation rates of *C. fetus* subsp. *jejuni*, of the other ducks ranged between those of green-winged teal and shovelers. Husson et al. (8) have shown that different species of waterfowl and also waterfowl of the same species maintained on different diets harbor substantially different total numbers of intestinal bacteria. It is, therefore, possible that dietary variation among the species studied could account for some of the differences in isolation rates of *C. fetus* subsp. *jejuni*.

The question was raised during the course of the study whether live birds could be trapped, cloacal swabs obtained, and the birds subsequently released unharmed. However, the isolation rates of *C. fetus* subsp. *jejuni* were so much higher from cecal specimens than from cloacal swabs that cecal samples were used throughout the study. Whether higher isolation rates from other species of animals would similarly be obtained by using cecal specimens instead of cloacal or rectal swabs is presently unknown.

Although *C. fetus* subsp. *jejuni* has been incriminated in avian hepatitis (a contagious disease of chickens characterized by diarrhea, weight loss, liver lesions, and drop in egg production) (7), none of the waterfowl in this study showed gross lesions of the liver or gut, and all were apparently healthy when collected. The high body temperature (42°C) of waterfowl and other birds may encourage carriage of this organism, whose optimum temperature for growth is also 42°C.

The sources of *C. fetus* subsp. *jejuni* for human infections remain to be fully elucidated. Campylobacters have been isolated from freshwater lakes and streams (10), and one large waterborne outbreak of *Campylobacter* infection occurred without a known source (18). Previous data from this laboratory showed that *Campylobacter* survives in stream water for over 1 week at 4°C (3). Waterfowl have been reported to occasionally harbor other enteric pathogens such as *Salmonella* (5, 12), and heavy concentrations of wild waterfowl can cause a significant elevation in fecal coliform counts of surface water and sediment (8). This study raises the possibility that migratory waterfowl may be yet another reservoir for *C. fetus* subsp. *jejuni*. Whether they contaminate freshwater ponds

and streams with this enteric pathogen or acquire the infection from water already polluted by the feces of domestic animals is not presently known.

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